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(54) Title
CONTRAST PREPARATION CONSISTING OF CAVITATE- OR CLATHRATE- FORMING HOST/GUEST COMPLEXES

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(56) Prior Art Documents
EP 224934
EP 28253

(57) Claim

1. A preparation for use as an injectible contrast agent in ultrasonic, X-ray or NMR investigations, said preparation comprising a pharmaceutically acceptable fluid vehicle and a cavitate- or clathrate-forming host/guest (h/g) complex which, when dissolved in said fluid vehicle releases the guest molecules from the host molecules as the host dissolves in said fluid vehicle, said guest molecules functioning as the contrast agent.

2. The preparation according to claim 1, wherein said host is selected from any one of water, urea and derivatives thereof, thiourea and derivatives thereof, phenol and substituted phenols, dihydroxybenzenes and derivatives thereof, hydro-quinone and substitute hydroquinones, salicylic acid and derivatives thereof, tri-*o*-thymotide and derivatives thereof, ascorbic acid, flavins and derivatives thereof, flavanols and derivatives thereof, cyclophane and derivatives thereof, guaiacamine, naphthohydro-quinones and derivatives thereof, chromanes and derivatives thereof, including 4-*p*-hydroxyphenyl-2,2,4-trimethylchromane, 4-*p*-hydroxyphenyl-2,2,4-trimethylthiochromane, 4-*p*-hydroxyphenyl-2,2,4,7-tetramethylthiochromane, 4-*p*-hydroxyphenyl-2,2,4-trimethylselenium chromane, hexahost compounds, including hexakis (phenylthio) benzene and derivatives thereof, cyclotriveratrylene and derivatives thereof, 1,1'-binaphthyl-2,2'-dicarboxylic acid and

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derivatives thereof, onium compounds and derivatives thereof, acetylsalicylic acid, di-, tri- and tetrasalicylides, 9,9'-spirobifluorene-2,2'-dicarboxylacid, choleic acids, 4,4'-dinitrodiphenyl, bis(N,N'-alkylenebenzidine), bis(N,N'-tetramethylenebenzidine), or desoxycholic acid, monoaminonickel (II) -cyanide, tetra- (4-methylpyridine) nickel (II) -dithiocyanatesandderivativesthereof,hexamethylisocyanidoferron-chlorides,2-phenyl-3-p-(2,2,4-trimethylchroman-4-yl)-phenylquinazoline-4, cyclotriphosphazone, tris-1,2-phenyldioxycyclotriphosphazones, or mixtures thereof, and said guest is selected from inert gases and inert gas compounds, sulphur halides, nitrogen and nitrogen oxides, carbon oxides, hydrogen and hydrogen oxides, sulphur oxides, hydrogen phosphides, hydrogen halides, uranium halides and oxygen as well as hydrocarbons and derivatives thereof, epoxides, ethers and halogenated hydrocarbons, or mixtures thereof.

12. A method for the preparation of an injectible contrast media which is to be used in ultrasonic, X-ray, or NMR investigations, said method comprising dissolving a cavitate- or clathrate- forming host/guest complex in a pharmaceutically acceptable fluid vehicle, the host by dissolving, releasing the guest which functions as a contrast agent.

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NOTICE OF ENTITLEMENT

SCHERING AKTIENGESELLSCHAFT, the applicant in respect of Application No 40651/89 state the following:

The Nominated Person is entitled to the grant of the patent because the Nominated Person derives title to the invention from the inventors.

The Nominated Peson is entitled to claim priority from the application listed in the declaration under Article 8 of the PCT because the Nominated Person made the application made the application listed in the declaration under Article 8 of the PCT, and because the application was the first application made in a convention country in respect of the invention.

DATED this 13th day of July 1993.

Peter Sleeman

a member of the firm of
DAVIES COLLISON CAVE for
and on behalf of the applicant.

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(54) Title: CONTRAST PREPARATION CONSISTING OF CAVITATE- OR CLATHRATE-FORMING HOST/GUEST COMPLEXES

(54) Bezeichnung: MITTEL BESTEHEND AUS CAVITATE ODER CLATHRATE BILDENDEN WIRT/GAST-KOMPLEXEN ALS KONTRASTMITTEL

(57) Abstract

The invention concerns the use of cavitate- or clathrate-forming host/guest complexes as contrast agents for ultrasonic, X-ray and NMR examinations.

(57) Zusammenfassung

Die Erfindung betrifft die Verwendung von Cavitate oder Clathrate bildenden Wirt/Gast-Komplexen als Kontrastmittel bei Ultraschall -, Röntgen- oder NMR-Untersuchungen.

Preparation comprising cavitate- or clathrat -forming host/guest complexes as contrast agent

The invention relates to a preparation comprising cavitate- or clathrate-forming host/guest complexes in accordance with the preamble of claim 1.

The manufacture of stoichiometric host/guest complexes comprising host molecules, significantly organic onium compounds and gases or gas formers as guest molecules has already been described in literature (Angew. Chem. 97 (1985) 721). Use of the host/guest complexes as contrast agents has not been described.

The invention is based on the problem of providing for ultrasonic, X-ray or NMR investigations a preparation which can be used as a transport medium for contrast agents. In particular the invention is to provide host/guest complexes which store the largest possible guest volume in a minimal host mass.

It has surprisingly been found that the cavitates or clathrates that are indicated in claim 1 form a transport medium which can completely decompose and can thus be chosen so that they do

not exert any toxic influence on the biological substance in which the investigation is to b carried out.

The preparation used for ultrasonic investigation can advantageously contain as host molecules

water, urea and derivatives thereof, thiourea and derivatives thereof, phenol and substituted phenols, dihydroxybenzenes and derivatives thereof, hydroquinone and substituted hydroquinones, salicyclic acid and derivatives thereof, tri-o-thymotide and derivatives thereof, ascorbic acid, flavins and derivatives thereof, flavanols and derivatives thereof, cyclophanes and derivatives thereof, guaiacamine, naphthohydroquinone and derivatives thereof, cyclodextrin and derivatives thereof, in particular dimethyl-1-cyclodextrin, methyl-β-cyclodextrin, hydroxypropyl-β-cyclodextrin, chromanes and derivatives thereof, in particular 4-p-hydroxyphenyl-2,2,4-trimethylchromane, 4-p-hydroxyphenyl-2,2,4-trimethyl-thiochromane, 4-p-hydrophenyl-2,2,4,7-tetramethylthiochromane, 4-p-hydroxyphenyl-2,2,4-trimethylselenium chromane, hexahost compounds, in particular hexakis(phenylthio)benzene and derivative thereof, cyclotriveratrylene and derivatives thereof, 1,1'-binaphthyl-2,2'-dicarboxylic acid and derivatives thereof,

onium compounds and derivatives thereof, acetylsalicylic acid, di-, tri- and tetra-salicylides, 9,9'-spirobifluorene-2,2'-dicarboxylacid, choleic acids, 4-4'dinitrodiphenyl, bis-(N,N'-alkylene-benzidine), bis-(N,N'-tetramethylene-benzidine), desoxycholic acid, monoaminonickel (II)-cyanide, tetra(4-methyl-pyridine)-nickel (II)-dithiocyanates and derivatives thereof, hexamethylisocyanidoferronchloride, 2-phenyl-3-p(2,2,4-trimethyl chroman —4-yl)-phenylquinazoline-4, cyclotriphosphazones, tris-1,2-phenyldioxycyclotriphosphazones

and as guest molecules:

inert gases and inert gas compounds, sulphur halides, nitrogen and nitrogen oxides, carbon oxides, hydrogen and hydrogen oxides, sulphur oxides, hydrogen phosphides, hydrogen halides, uranium halides and oxygen as well as hydrocarbons and derivatives thereof, epoxides, ether and halogenated hydrocarbons

The preparation used for ultrasonic investigation can especially advantageously contain as guest molecules helium, neon, argon, krypton, xenon, radon, sulphur hexafluoride, water, hydrogen peroxide, nitrogen monoxide, carbon monoxide, carbon dioxide, hydrogen iodide, xenon difluoride, xenon

tetrafluoride, xenonhexafluoride, xenon dioxide, sulphur dioxide, sulphur trioxide, arsenic hydride, hydrogen phosphide, deuterium, uranium hexafluoride, methane, ethane, propane, cyclopropane, butane, pentane, ethylene oxide and methyl bromide.

The crystalline complexes can be influenced in their particle size in particular by the crystallisation conditions and also by the mechanical processes of the particle breakdown (air jet grinding).

The crystalline complexes can be coated with hydrophilic , lipophilic or amphiphilic auxiliary products.

Suitable vehicles for applying the complexes are sterile aqueous systems with additives to adjust the viscosity, surface tension, pH-value and osmotic pressure wherein the complexes are dissolved, or suspended and optionally emulsified preferably prior to use.

The host/guest complexes are introduced into an aqueous vehicle. As the host molecules dissolve the complexes are broken down through the release of the gas bubbles into the vehicle.

The host molecules dissolved in the vehicle no longer have any complexing properties. The speed of the gas release, and the size and duration of the gas bubbles can be adjusted within a wide range through the type of gas or gas-former enclosed, through the type of host molecule and by the surface or particle size in dependence on the viscosity, surface tension of the vehicle.

It is thus surprisingly possible to obtain in a very simple way injectable, gas-containing pharmaceutical preparations with excellent echogenic properties.

In particular it is possible to prepare the gas volume of about 150 µl required for in vivo contrasting eg of the left ventricle of a human being through very low amounts of active ingredient in the range from 2 - lo mg/appln., as shown by the following composition:

Hydroquinone/N_2	3:1	Complex	1 mg	70 µl
Hydroquinone/Xe	3:1	Complex	1 mg	53 µl
Dianin/SF_6	3:1	"	1 mg	26 µl
Dianin/Argon	2:1	"	1 mg	26 µl
Tri-o-thymotide/methane	2:1	"	1 mg	23 µl
Tri-o-thymotide CH_3Br	2:1	"	1 mg	21 µl
Dianin/N2			1 mg	103 µl

4-(4-hydroxyphenyl)-2,2,4-trimethyl-chromane) is named as the dianin compound and produced according to J. Russ Phys. Chem. Soc. 46,1310 (1914) and Chem. Zentr. 1915,I,1063.

It is thus possible to prepare a contrast agent for ultrasonic diagnostics which after intravenous application is able to show up the blood and its flow conditions on the right side of the heart and after passing through the pulmonary capillary bed on the left side for ultrasound. Furthermore it also is to show the circulation to other organs, such as the myocardium, liver, spleen and kidneys. It can similarly be used to show the urinary ducts, gastro-intestinal tract, joints, frontal sinus and eyes.

Particularly when using gas molecules (eg xenon) which are able to overcome the blood/brain barrier, it is also possible to show the cerebrum and its physiological and pathological structures through ultrasound.

If the preparation according to the invention also contains eg xenon then it is possible to use this host/guest complex as an X-ray contrast agent. When using stable radicals (eg oxygen-, nitroxyl-) the preparations according to the invention can also be used as NMR-contrast agents.

The invention will now be explained by the following examples.

1. Tri-o-thymotide/methyl bromide

Tri-o-thymotide (25g) was dissolved in 2,2,4-trimethylpentane (50ml) at loo °C and the hot solution was introduced into the high pressure autoclave. Methyl bromide was added to the autoclave until a pressure of 200 bar was reached. The high pressure autoclave was then kept for 2 hours at 110°C and the solution was then cooled down to room temperature within 5 days. The crystals were filtered off and washed 3 times with cold 2,2,4-trimethylpentane. The crystals were then dried in the drying cabinet at 50°C.

2. Dianin-compound (4-p-hydroxyphenyl-2,2,4-trimethylchromane)/ ethylene oxide

Dianin compound (25g) was dissolved in 1-decanol (35 g) at 125° C. The hot solution was introduced into the high pressure autoclave. The solution was subjected to compressed ethylene oxide of 3oo bar. The high pressure autoclave was kept for 2 hours at 140°C and the solution then cooled down to room temperature

within 8 days. The crystals were filtered off and washed 4 times with cold 1-decanol (5ml). The crystals were then dried in the drying cabinet at 100°C.

3. Dianin-compound (4-p-hydroxyphenyl)-2,2,4-trimethyl-chromane/sulphur hexafluoride

Dianin compound (25g) was dissolved in 1-decanol (35g) at 125°C. The hot solution was introduced into the high pressure autoclave. The solution was subjected to compressed sulphur hexafluoride of 300 bar. The high pressure autoclave was tempered for 2 hours at 140°C. The solution was then cooled down to room temperature within 8 days. The crystals were filtered off and washed 4 times with cold 1-decanol (5ml). The crystals were subsequently dried in the drying cabinet at 100°C.

4. Dianin-compound (4-p-hydroxyphenyl-2,2,4-trimethyl-chromane)/ethane

Dianin-compound (25 g) was dissolved in 1-decanol (35g) at 125°C. The hot solution was introduced into the high pressure autoclave. The solution was subjected to compressed ethane

of 300 bar. The high pressure autoclave was kept for 2 h at 140°C. The solution was then cooled down to room temperature within 8 days. The crystals were filtered off and washed 4 times with cold 1-decanol (5ml). Then the crystals were dried in the drying cabinet at 100°C.

5. Dianin-compound (4-p-hydroxyphenyl-2,2,4-trimethyl-chromane)/propane

Dianin compound (25g) was dissolved in 1-decanol (35 g) at 125°C. The hot solution was introduced into the high pressure autoclave. The solution was subjected to compressed propane of 300 bar. The high pressure autoclave was kept for 2 h at 140°C. The solution was then cooled down to room temperature within 8 days. The crystals were filtered off and washed 4 times with cold 1-decanol (5 ml). The crystals were then dried in the drying cabinet at 100°C.

6. Dianin-compound (4-p-hydroxyphenyl-2,2,4-trimethyl-chromane)/carbon dioxide

Dianin-compound (25 g) was dissolved in 1-decanol (35g)

at 125°C. The hot solution was introduced into the high pressure autoclave. The solution was subjected to compressed carbon dioxide of 300 bar. The high pressure autoclave was kept for 2 h at 140°C. The solution was then cooled down to room temperature within 8 days. The crystals were filtered off and washed 4 times with cold 1-decanol (5 ml). The crystals were then dried in the drying cabinet at 100°C.

7. Dianin-compound (4-p-hydroxyphenyl-2,2,4-trimethyl-chromane/cyclopropane

Dianin-compound (25 g) was dissolved in 1-decanol (35 g) at 125°C. The hot solution was introduced into the high pressure autoclave. The solution was subjected to compressed cyclopropane of 300 bar. The high pressure autoclave was kept for 2 h at 140°C. The solution was then cooled down to room temperature within 8 days. The crystals were filtered off and washed 4 times with cold 1-decanol (5 ml). The crystals were dried in the drying cabinet at 100°C.

8. Dianin-compound (4-p-hydroxyphenyl-2,2,4-trimethyl-chromane)/methane

Dianin-compound (25 g) was dissolved in 1-d canol (35 g) at

125°C. The hot solution was introduced into the high pressure autoclave. The solution was subjected to compressed methane of 300 bar. The high pressure autoclave was kept for 2 h at 140°C. The solution was then cooled down to room temperature within 8 days. The crystals were filtered off and washed 4 times with cold 1-decanol (5 ml). The crystals were dried in the drying cabinet at 100°C.

9. Dianin-compound (4-p-hydroxyphenyl-2,2,4-trimethyl-chromane)/nitrogen

Dianin-compound (25 g) was dissolved in 1-decanol (35 g) at 125°C. The hot solution was introduced into the high pressure autoclave. The solution was subjected to compressed nitrogen of 300 bar. The high pressure autoclave was kept for 2 h at 140°C. The solution was then cooled down to room temperature within 8 days. The crystals were filtered off and washed 4 times with cold 1-decanol (5 ml). The crystals were then dried in the drying cabinet at 100°C.
Melting point: 162.88°C.

10. Dianin-compound (4-p-hydroxyphenyl-2,2,4-trimethyl-chromane)/xenon

Dianin-compound (25 g) was dissolved in 1-decanol (35 g) at 125°C. The hot solution was introduced into the high pressure autoclave. The solution was subjected to compressed xenon of 300 bar. The high pressure autoclave was kept for 2 h at 140°C. The solution was then cooled down to room temperature within 8 days. The crystals were filtered off and washed 4 times with cold 1-decanol (5 ml). The crystals were then dried in the drying cabinet at 100°C.

11. Dianin-compound (4-p-hydroxyphenyl-2,2,4-trimethyl-chromane)/argon

Dianin-compound (25 g) was dissolved in 1-decanol (35 g) at 125°C. The hot solution was introduced into the high pressure autoclave. The solution was subjected to compressed argon of 300 bar. The high pressure autoclave was kept for 2 h at 140°C. The solution was then cooled down to room temperature within 8 days. The crystals were filtered off and washed 4 times with cold 1-decanol (5 ml). The

crystals were then dried in the drying cabinet at 100°C. Melting point: 160.84°C.

12. Hydroquinone/methane

Hydroquinone (30 g) was dissolved in n-propanol (70 ml) at 70°C. The hot solution was introduced into the high pressure autoclave. The solution was subjected to compressed methane of 300 bar. The high pressure autoclave was kept for 2 h at 80°C. The solution was then cooled down to room temperature within 5 days. The crystals were filtered off and then washed 4 times with cold n-propanol (5 ml). The crystals were dried in the drying cabinet at 70°C subsequently.

13. Hydroquinone/sulphur hexafluoride

Hydroquinone (30 g) was dissolved in n-propanol (70 ml) at 70°C. The hot solution was introduced into the high pressure autoclave. The solution was subjected to compressed sulphur

hexafluoride of 300 bar. The high pressure autoclave was kept for 2 h at 80°C. The solution was then cooled down to room temperature within 5 days. The crystals were filtered off and washed 4 times with cold n-propanol (5 ml). The crystals were then dried in the drying cabinet at 70°C.

14. Hydroquinone/propane

Hydroquinone (3o g) was dissolved in n-propanol (70 ml at 70°C. The hot solution was introduced into the high pressure autoclave. The solution was subjected to compressed propane of 3oo bar. The high pressure autoclave was kept for 2 h at 80°C. The solution was then cooled down to room temperature within 5 days. The crystals were filtered off and washed 4 times with cold n-propanol (5 ml). The crystals were then dried in the drying cabinet at 70°C.

15. Hydroquinone/ethane

Hydroquinone (30 g) was dissolved in n-propanol (70 ml) at 70°C. The hot solution was introduced into the high

pressure autoclave. The solution was subjected to compressed ethane of 300 bar. The high pressure autoclave was kept for 2 h at 80°C. The solution was then cooled down to room temperature within 5 days. The crystals were filtered off and washed 4 times with cold n-propanol (5 ml). Then the crystals were dried in the drying cabinet at 70°C.

16. Hydroquinone/carbon dioxide

Hydroquinone (30 g) was dissolved in n-propanol (70 ml) at 70°C. The hot solution was introduced into the high pressure autoclave. The solution was subjected to compressed carbon dioxide of 300 bar. The high pressure autoclave was kept for 2 h at 80°C. Then the solution was cooled down to room temperature within 5 days. The crystals were filtered off and washed 4 times with cold n-propanol (5 ml). The crystals were then dried in the drying cabinet at 70°C.

17. Hydroquinone/ethylene oxide

Hydroquinone (30g) was dissolved in n-propanol (70 ml) at 70°C.

The hot solution was introduced into the high pressure autoclave. The solution was subjected to compressed ethylene oxide of 300 bar. The high pressure autoclave was kept for 2 h at 80°C. The solution was then cooled down to room temperature within 5 days. The crystals were filtered off and washed 4 times with cold n-propanol (5 ml). The crystals were then dried in the drying cabinet at 70°C.

18. Hydroquinone/cyclopropane

Hydroquinone (30 g) was dissolved in n-propanol (70 ml) at 70°C. The hot solution was introduced into the high pressure autoclave. The solution was subjected to compressed cyclopropane of 300 bar. The high pressure autoclave was kept for 2 h at 80°C. The solution was then cooled down to room temperature within 5 days. The crystals were filtered off and washed 4 times with cold n-propanol (5 ml). The crystals were then dried in the drying cabinet at 70°C.

19. Hydroquinone/nitrogen

Hydroquinone (30g) was dissolved in n-propanol (70 ml) at 70°C. The hot solution was introduced into the high pressure autoclave. The solution was subjected to compressed nitrogen of 300 bar. The high pressure autoclave was kept for 2 h at 80°C. The solution was then cooled down to room temperature within 5 days. The crystals were filtered off and washed 4 times with cold n-propanol (5 ml). The crystals were dried in the drying cabinet thereafter at 70° C.

Melting point : 176.92°C.

20. Hydroquinone/xenon

Hydroquinone (30g) was dissolved in n-propanol (70 ml) at 70°C. The hot solution was introduced into the high pressure autoclave. The solution was subjected to compressed xenon of 300 bar. The high pressure autoclave was kept for 2 h at 80°C . The solution was then cooled down to room temperature within 5 days. The crystals were filtered off and washed 4 times with cold n-propanol (5 ml). The crystals were then dried in the drying cabinet at 70°C.

21. Hydroquinone/argon

Hydroquinone (30 g) was dissolved in n-propanol (70 ml) at 70°C. The hot solution was placed in the high pressure autoclave. The solution was subjected to compressed argon of 300 bar. The high pressure autoclave was kept at 80°C for 2 h . The solution was then cooled down to room temperature within 5 days. The crystals were filtered off and washed 4 times with cold n-propanol (5 ml). The crystals were then dried in the drying cabinet at 70°C.
Melting point : 175.67°C.

22. Urea/butane

4 g urea were dissolved in 12 ml ethanol at 60°C. The solution was then placed in an high pressure autoclave and subjected to a butane pressure of 150 bar. The solution was cooled down from 60°C to room temperature within 48 h. The solution with h/g crystals was removed from the autoclave, filtered and the h/g crystals were washed with 10 ml cold ethanol. The h/g complex crystals were dried in the vacuum cabinet at 60°C.

23. Urea/isobutane

4 g urea were dissolved in 12 ml ethanol at 60°C. The solution was then placed in a high pressure autoclave and subjected to an isobutane pressure of 150 bar. The solution was cooled down from 60°C to room temperature within 48 h. The solution with h/g crystals was removed from the autoclave, filtered and the h/g crystals were washed with 10 ml cold ethanol. The h/g complex cystals were dried in the vacuum cabinet at 60°C.
Melting point: 138.50 °C.

24. Urea/neopentane

4 g urea were dissolved in 12 ml ethanol at 60°C. The solution was then placed in a high pressure autoclave and subjected to a neopentane pressure of 150 bar. The solution was cooled down from 60°C to room temperature within 48 h. The solution with h/g crystals was removed from the autoclave, filtered and the h/g crystals were washed with 10 ml cold ethanol. The h/g complex crystals were dried in the vacuum cabinet at 60°C.
Melting point: 138.79°C.

25. Thiourea/butane

4 g thiourea were dissolved in 12 ml ethanol at 60°C. The solution was then placed in a high pressure autoclave and subjected to a butane pressure of 150 bar. The solution was cooled down to room temperature within 60 h. The solution with h/g crystals was removed from the autoclave, filtered and the h/g crystals were washed with 10 ml cold ethanol. The h/g complex crystals were dried in the vacuum cabinet at 60°C.

26. Thiourea/isobutane

4 g thio urea were dissolved in 20 ml ethanol at 60°C. The solution was then placed in a high pressure autoclave and subjected to an isobutane pressure of 150 bar. The solution was cooled down to room temperature within 60 h. The solution with h/g crystals was removed from the autoclave, filtered and the h/g crystals were washed with 10 ml cold ethanol. The h/g complex crystals were dried in the vacuum cabinet at 60°C.

Melting point : 181.34°C.

27. Thiourea/neopentane

4 g thiourea were dissolved in 20 ml ethanol at 60°C. The solution was then placed in a high pressure autoclave and subjected to a neopentane pressure of 150 bar. The solution was cooled down to room temperature within 60 h. The solution with h/g crystals was removed from the autoclave, filtered and the h/g crystals were washed with 10 ml cold ethanol. The h/g complex crystals were dried in the vacuum cabinet at 60°C.

28. Vehicle

A: The following solutions for example are suitable as a vehicle for hydroquinone-, tri-O-thymotide-urea- and thiourea-h/g complexes:

a) 1 % gelatine solution
b) 1 % albumin solution
c) 10 % glycerin solution
d) 15 % propylene glycol solution
e) Mixtures of sodium cholate and phosphatidylcholine in water
f) 0.01 - 1 % phosphatidylcholine dispersion (aqueous)
g) 1 % methyl cellulose
h) 1 - 2 % dextran solution

i) 1 % agar solution

j) 2 % "Tween" solution (Tween 80)

k) 1 % gum arabic

B: The following vehicles are suitable for dianin-h/g-complexes, for example:

a) 10 - 20 % 2-(2-methoxyethoxy)-ethanol

b) Mixtures of 2 - (2 methoxyethoxy)-ethanol (20 %) and 'Tween' 80 (1 %)

In vitro ultrasonic investigations

The acoustic properties of the h/g complex-vehicle systems were determined with in-vitro ultrasonic investigations.

For this about 1 - 5 mg of the h/g complexes were mixed in 10 - 20 ml with one of the said vehicles and then examined with ultrasonic scanners.

The ultrasonic scanner Ekoline 20A/S was used in the frequency range 1 - 5 MHz for qualitative examinations.

Quantitative measurements of the acoustic properties were obtained in an apparatus with the ultrasonic scanner Kraut-Kraemer U.S.I. P-12 at 4 MHz. The results of four systems are detailed here by way of example (Figs. 1 - 4).

Fig. 1: Urea/isobutane (Example 23) in 2 % 'Tween' 80 solution

Fig. 2: Thiourea/isobutane (Example 26) in 1% dextran solution

Fig. 3: Hydroquinone/argon (Example 21) in 1 % gelatine solution

Fig. 4: Dianin/argon (Example 11) in 10 % 2 (2-methoxyethoxy)-ethanol

To explain the ultrasonic measuring apparatus and the diagrams obtained therefrom:

The apparatus comprises an ultrasonic transmitter combined with a receiver and measuring bulb which contains the specimen. An ultrasonic impulse is transmitted to measure the acoustic properties of the specimen. Reflected ultrasound is measured

by the receiver and indicated through a change in the amplitude (see diagram). The diagrams each only show one amplitude change which results from the reflection of the ultrasound from the front wall of the measuring bulb. A second amplitude change which results from reflection from the back wall of the measuring bulb is only obtained with non-echogenic substances (eg water). In the case of echogenic substances a second reflected signal is not obtained since the ultrasound is dissipated in the specimen or changed so that it can no longer be received.

The claims defining the invention are as follows:

1. A preparation for use as an injectible contrast agent in ultrasonic, X-ray or NMR investigations, said preparation comprising a pharmaceutically acceptable fluid vehicle and a cavitate- or clathrate-forming host/guest (h/g) complex which, when dissolved in said fluid vehicle releases the guest molecules from the host molecules as the host dissolves in said fluid vehicle, said guest molecules functioning as the contrast agent.

2. The preparation according to claim 1, wherein said host is selected from any one of water, urea and derivatives thereof, thiourea and derivatives thereof, phenol and substituted phenols, dihydroxybenzenes and derivatives thereof, hydro-quinone and substitute hydroquinones, salicylic acid and derivatives thereof, tri-*o*-thymotide and derivatives thereof, ascorbic acid, flavins and derivatives thereof, flavanols and derivatives thereof, cyclophane and derivatives thereof, guaiacamine, naphthohydro-quinones and derivatives thereof, chromanes and derivatives thereof, including 4-*p*-hydroxyphenyl-2,2,4-trimethylchromane, 4-*p*-hydroxyphenyl-2,2,4-trimethylthiochromane, 4-*p*-hydroxyphenyl-2,2,4,7-tetramethylthiochromane, 4-*p*-hydroxyphenyl-2,2,4-trimethylselenium chromane, hexahost compounds, including hexakis (phenylthio) benzene and derivatives thereof, cyclotriveratrylene and derivatives thereof, 1,1'-binaphthyl-2,2'-dicarboxylic acid and derivatives thereof, onium compounds and derivatives thereof, acetylsalicylic acid, di-, tri- and tetrasalicylides, 9,9'-spirobifluorene-2,2'-dicarboxylacid, choleic acids, 4,4'-dinitrodiphenyl, bis(N,N'-alkylenebenzidine), bis(N,N'-tetramethylenebenzidine), or desoxycholic acid, monoaminonickel (II) -cyanide, tetra- (4-methylpyridine) nickel (II) - dithiocyanatesandderivativesthereof, hexamethylisocyanidoferron-chlorides, 2-phenyl-3-p-(2,2,4-trimethylchroman-4-yl)-phenylquinazoline-4, cyclotriphosphazone, tris-1,2-phenyldioxycyclotriphosphazones, or mixtures thereof, and said guest is selected from inert gases and inert gas compounds, sulphur halides, nitrogen and nitrogen oxides, carbon oxides, hydrogen and hydrogen oxides, sulphur oxides, hydrogen phosphides, hydrogen halides, uranium halides and oxygen as well as hydrocarbons and derivatives thereof, epoxides, ethers and halogenated hydrocarbons, or mixtures thereof.

3. The preparation according to claim 1 or claim 2 wherein said host so selected from any one of:

hydroquinone, dianin, urea, thiourea, or tri-*o*-thymotide.

4. The preparation according to any one of claims 1 to 3, wherein said guest is selected from any one of:

helium, neon, argon, krypton, xenon, radon, sulfur hexafluoride, water, hydrogen peroxide, nitrous oxide, carbon monoxide, carbon dioxide, hydrogen iodide, xenon difluoride, xenon tetrafluoride, xenonhexafluoride, xenon dioxide, sulfur dioxide, sulfur trioxide, arsenic hydride, hydrogen phosphide, deuterium, uranium hexafluoride, methane, ethane, propane, cyclopropane, butane, pentane, and the isomers thereof, ethylene oxide and methyl bromide or mixtures thereof.

5. The preparation according to claim 4 particularly for use in ultrasonic investigations whereby said guest is selected from any one of:

nitrogen, xenon, argon, sulfur hexafluoride, methane, ethane, propane, butane, isobutane, pentane, neopentane, cyclopropane, methylbromide, ethyleneoxide, carbon dioxide, or the mixtures thereof.

6. The preparation according to claim 4 particularly for use in X-ray investigations. whereby said guest is xenon.

7. The preparation according to claim 4 particularly fur use in NMR investigations, whereby said guest is selected from oxygen or nitrous oxide.

8. The preparation according to any one of claims 1 to 7 wherein said fluid vehicle is primarily sterile water or 2-(2-methoxyethoxy)-ethanol.

9. The preparation according to claim 8 which contains one or more additives to adjust the viscosity, surface tension, pH, or osmotic pressure of the preparation.

10. The preparation according to claim 9, wherein said additive is selected from any one or more of:

gelatin, albumin, glycerin, propylene glycol, sodium cholate, phosphatidylcholine, methyl cellulose, dextran, agar, a surfactant, gum arabic, or 2-(2-methoxyethoxy)-ethanol.

11. A preparation for use as an injectable contrast agent in ultrasonic, X-ray or NMR investigations, substantially as herein described with reference to any one of Examples 1 to 27.

12. A method for the preparation of an injectible contrast media which is to be used in ultrasonic, X-ray, or NMR investigations, said method comprising dissolving a cavitate- or clathrate- forming host/guest complex in a pharmaceutically acceptable fluid vehicle, the host by dissolving, releasing the guest which functions as a contrast agent.

13. The method according to claim 12 wherein said host is selected from any one of water, urea and derivatives thereof, thiourea and derivatives thereof, phenol and substituted phenols, dihydroxybenzenes and derivatives thereof, hydro-quinone and substitute hydroquinones, salicylic acid and derivatives thereof, tri-*o*-thymotide and derivatives thereof, ascorbic acid, flavins and derivatives thereof, flavanols and derivatives thereof, cyclophane and derivatives thereof, guaiacamine, naphthohydro-quinones and derivatives thereof, chromanes and derivatives thereof, more particularly 4-*p*-hydroxyphenyl-2,2,4-trimethylchromane, 4-*p*-hydroxyphenyl-2,2,4-trimethylthiochromane, 4-*p*-hydroxyphenyl-2,2,4,7-tetramethylthiochromane, 4-*p*-hydroxyphenyl-2,2,4-trimethylselenium chromane, hexahost compounds, more particularly hexakis (phenylthio) benzene and derivatives thereof, cyclotriveratrylene and derivatives thereof, 1,1'-binaphthyl-2,2'-dicarboxylic acid and derivatives thereof, onium compounds and derivatives thereof, acetylsalicylic acid, di-, tri- and tetrasalicylides, 9,9'-spirobifluorene-2,2'-dicarboxylacid, choleic acids, 4,4'-dinitrodiphenyl, bis(N,N'-alkylenebenzidine), bis(N,N'-tetramethylenebenzidine), desoxycholic acid, monoaminonickel (II) -cyanide, tetra- (4-methylpyridine) nickel (II) -dithiocyanatesandderivativesthereof, hexamethylisocyanidoferron-chlorides, 2-phenyl-3-p-

(2,2,4-trimethylchroman-4-yl)-phenylquinazoline-4, cyclotriphosphazone, tris-1,2-phenyldioxycy[illegible]iphosphazones, or mixtures thereof, and said guest is selected from inert gases and in[illegible] compounds, sulphur halides, nitrogen and nitrogen oxides, carbon oxides, hydrogen and hydrogen oxides, sulphur oxides, hydrogen phosphides, hydrogen halides, uranium halides and oxygen as well as hydrocarbons and derivatives thereof, epoxides, ethers and halogenated hydrocarbons, or mixtures thereof.

14. The method according to claim 13 said host so selected from any one of: hydroquinone, dianin, urea, thiourea, or tri-*o*-thymotide.

15. The method according to claim 14 wherein said guest is selected from any one of: helium, neon, argon, krypton, xenon, radon, sulfur hexafluoride, water, hydrogen peroxide, nitrous oxide, carbon monoxide, carbon dioxide, hydrogen iodide, xenon difluoride, xenon tetrafluoride, xenonhexafluoride, xenon dioxide, sulfur dioxide, sulfur trioxide, arsenic hydride, hydrogen phosphide, deuterium, uranium hexafluoride, methane, ethane, propane, cyclopropane, butane, pentane, and the isomers thereof, ethylene oxide and methyl bromide or mixtures thereof.

16. The method according to claim 15 particularly for use in ultrasonic investigations whereby said guest is selected from any one of:

nitrogen, xenon, argon, sulfur hexafluoride, methane, ethane, propane, butane, isobutane, pentane, neopentane, cyclopropane, methylbromide, ethyleneoxide, carbon dioxide, or the mixtures thereof.

17. The method according to claim 16 particularly for use in X-ray investigations. whereby said guest is xenon.

18. The method according to claim 17 particularly for use in NMR investigations,

whereby said guest is selected from oxygen or nitrous oxide.

19. The method according to claim 18 wherein said fluid vehicle is primarily sterile water or 2-(2-methoxyethoxy)-ethanol.

20. The method according to claim 19 which contains one or more additives to adjust the viscosity, surface tension, pH, or osmotic pressure of the preparation.

21. The method according to claim 20 wherein said additive is selected from any one or more of:

gelatin, albumin, glycerin, propylene glycol, sodium cholate, phosphatidylcholine, methyl cellulose, dextran, agar, a surfactant, gum arabic, or 2-(2-methoxyethoxy)-ethanol.

22. A method for the preparation of an injectible contrast media, said method substantially as herein described with reference to the "in vitro ultrasonic investigation" example and its associated drawings.

23. A method for conducting an ultrasonic, X-ray or NMR investigation of a subject using a contrast agent, characterized in that a preparation according to any one of claims 1 to 11 is prepared and injected into said subject at a suitable location in said subject and in a sufficient amount to provide contrast.

DATED this 13day of July 1993.

SCHERING AKTIENGESELLSCHAFT
By their Patent Attorneys
DAVIES COLLISON CAVE

350537/D#4/vmj

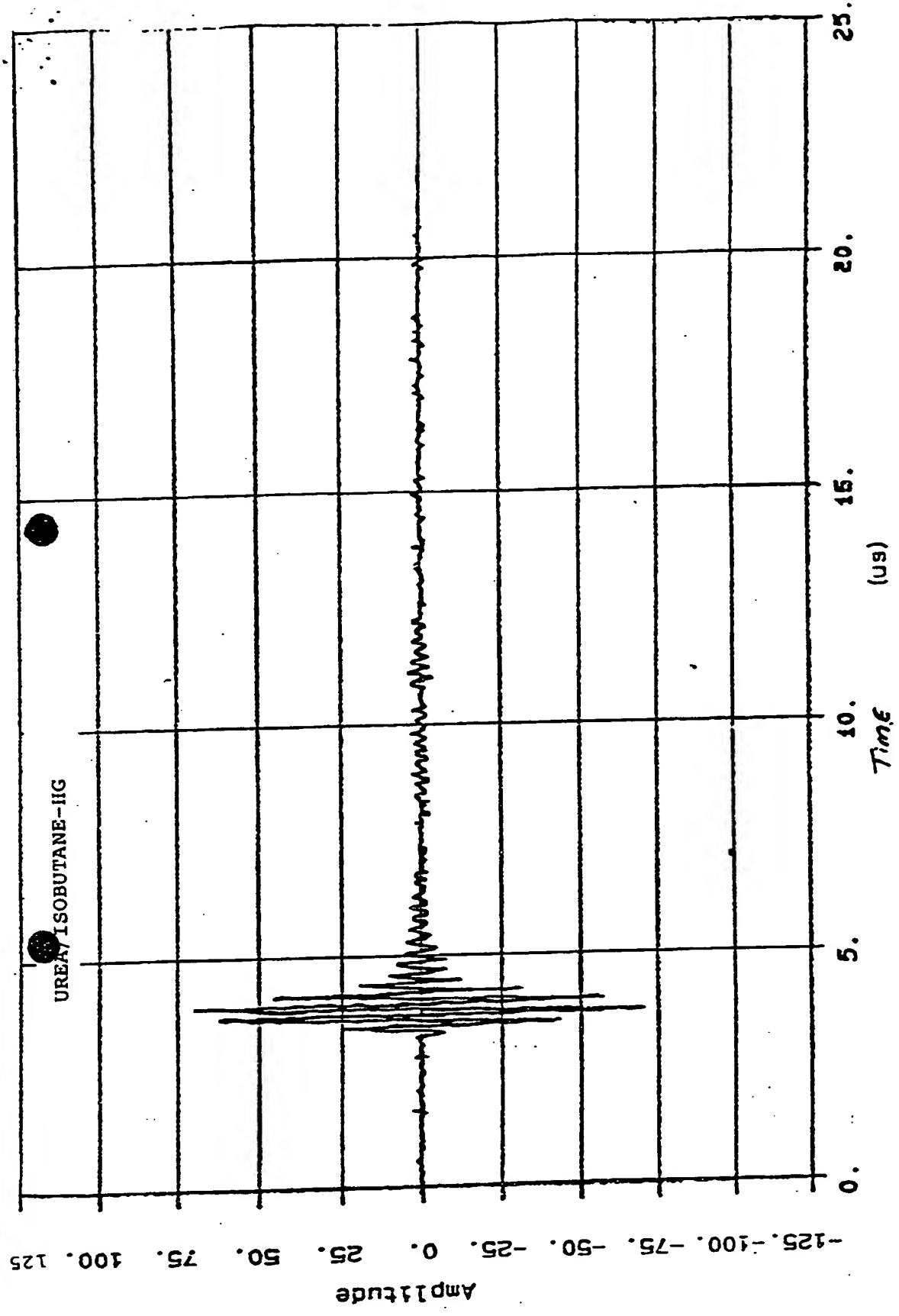
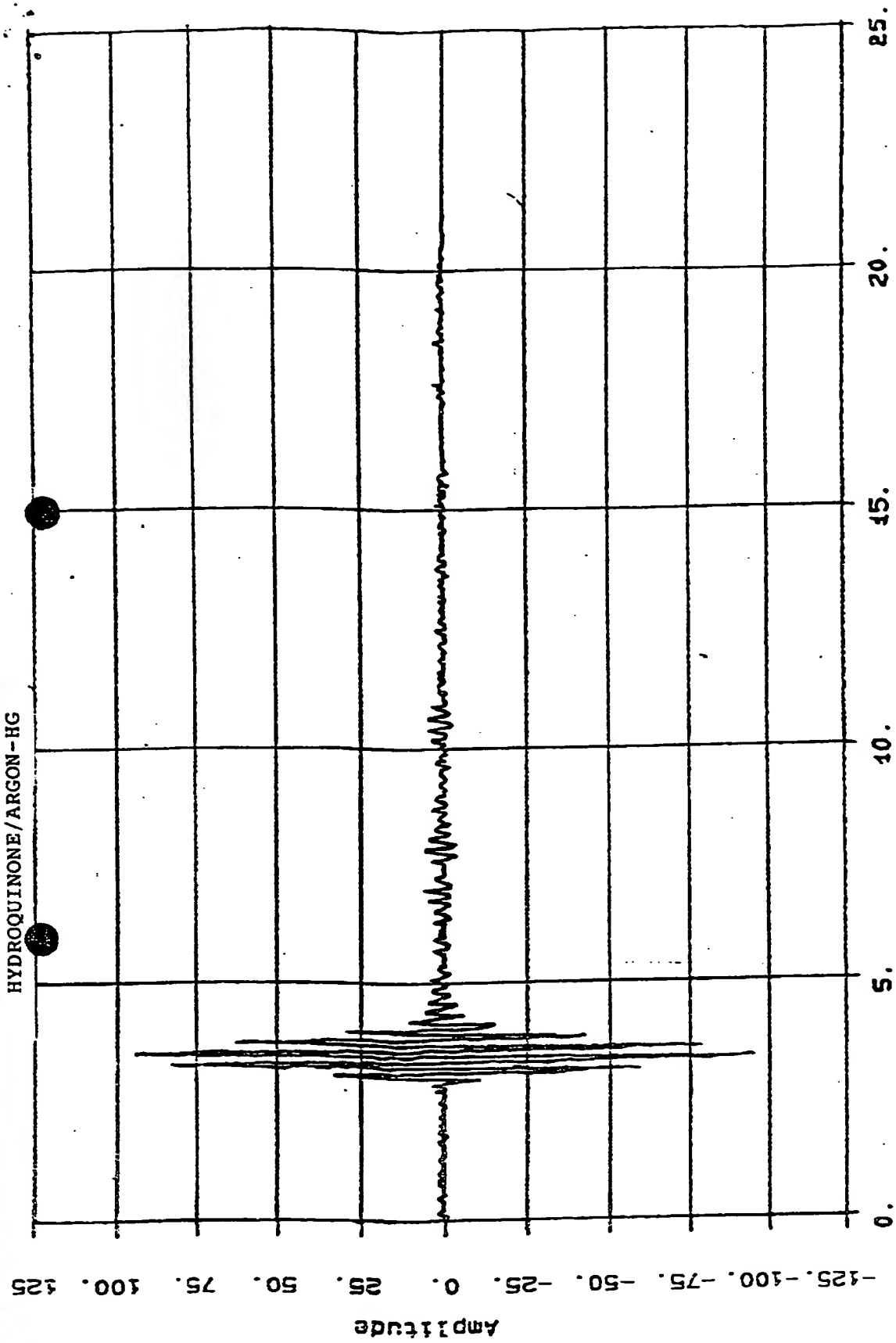

UREA/ISOBUTANE-HG
Amplitude
-125. -100. -75. -50. -25. 0. 25. 50. 75. 100. 125
0. 5. 10. 15. 20. 25.
Time (us)

Fig. 1

THIOUREA/ISOBUTANE-HG
Amplitude
-125. -100. -75. -50. -25. 0. 25. 50. 75. 100. 125.
0. 5. 10. 15. 20. 25.
Time (us)

Fig. 2


HYDROQUINONE/ARGON-HG
Amplitude
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0. 5. 10. 15. 20. 25.
Time (us)

Fig. 3

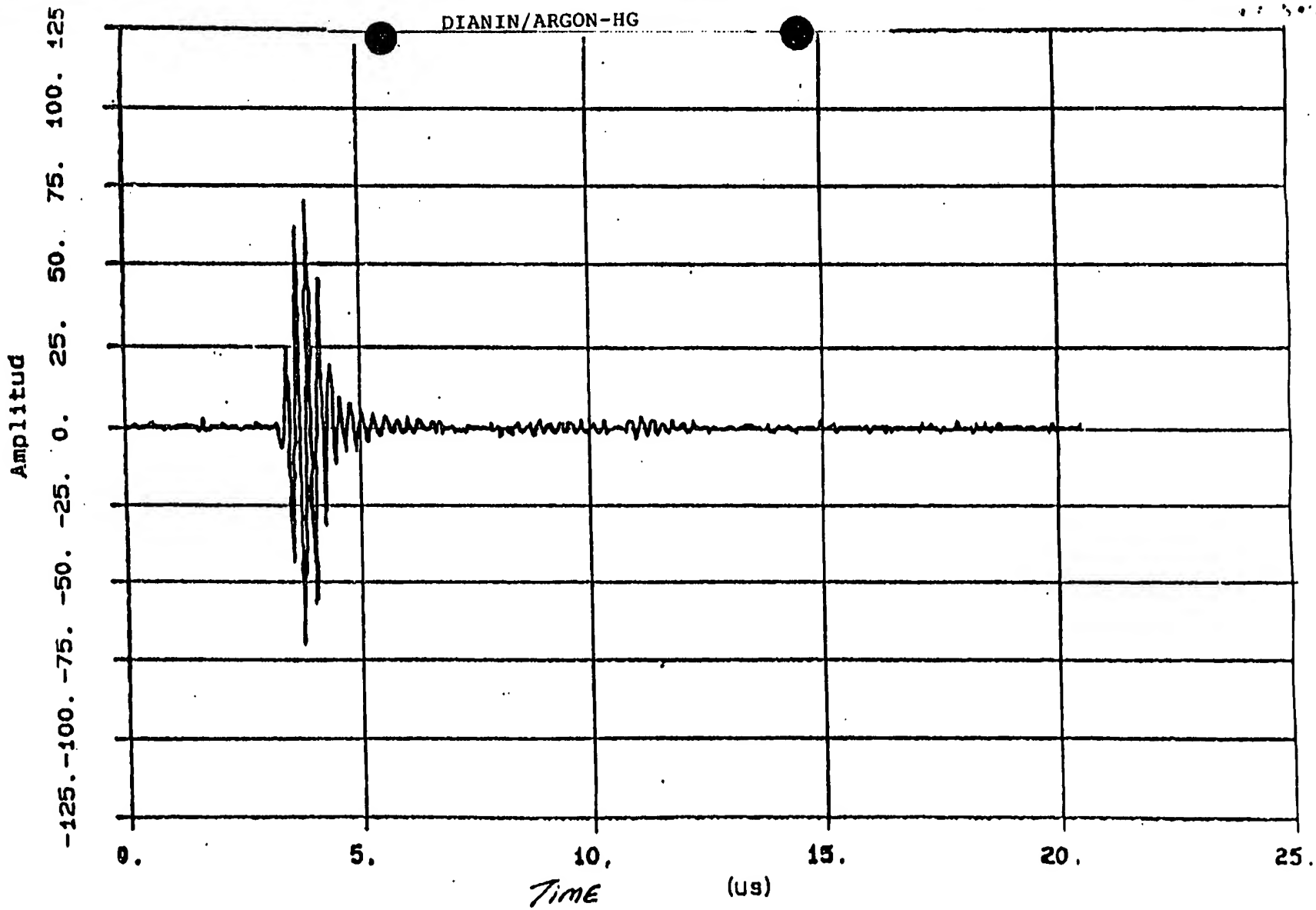

Fig. 4

INTERNATIONAL SEARCH REPORT

International Application No PCT/DE 89/00548

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) *

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl[5] A 61 K 49/00, A 61 K 49/04

II. FIELDS SEARCHED

Minimum Documentation Searched *

Classification System	Classification Symbols
Int.Cl[5]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched *

III. DOCUMENTS CONSIDERED TO BE RELEVANT *

Category *	Citation of Document, ** with indication, where appropriate, of the relevant passages **	Relevant to Claim No. **
A	Patent Abstracts of Japan, volume 5, No. 160 (C-75)(832), 15 October 1981, & JP, A, 5692221 (ZERIA SHINYAKU KOGYO K.K.) 25 july 1981 --	
A	WO, A, 80/02365 (RASOR ASS. INC.) 13 November 1980 --	
A	EP, A, 0224934 (FEINSTEIN) 10 June 1987 --	
A	DE, A, 3637926 (SCHERING AG) 26 november 1987	

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* Special categories of cited documents: **

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
21 November 1989 (21.11.89)	10 January 1990 (10.01.90)
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	

Form PCT/ISA/210 (second sheet) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. DE 8900548 SA 30565

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 19/12/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A- 8002365	13-11-80	US-A-	4276885	07-07-81
		AU-A-	6053580	20-11-80
		CA-A-	1171952	31-07-84
		EP-A-	0028253	13-05-81
EP-A- 0224934	10-06-87	US-A-	4718433	12-01-88
		AU-B-	575735	04-08-88
		AU-A-	6609786	11-06-87
		JP-A-	62181033	08-08-87
		US-A-	4774958	04-10-88
DE-A- 3637926	26-11-87	WO-A-	8803388	19-05-88
		EP-A-	0273140	06-07-88
		EP-A-	0296189	28-12-88

INTERNATIONALER RECHERCHENBERICHT

Internationales Aktenzeichen: PCT/DE 89/00548

I. KLASSIFIKATION DES ANMELDUNGSGEGENSTANDS (bei mehreren Klassifikationssymbolen sind alle anzugeben)[6]

Nach der Internationalen Patentklassifikation (IPC) oder nach der nationalen Klassifikation und der IPC

Int C: 5: A 61 K 49/00, A 61 K 49/04

II. RECHERCHIERTE SACHGEBIETE

Recherchierter Mindestprüfstoff[7]

Klassifikationssystem	Klassifikationssymbole
Int. Cl 5	A 61 K

Recherchierte nicht zum Mindestprüfstoff gehörende Veröffentlichungen, soweit diese unter die recherchierten Sachgebiete fallen[8]

III. EINSCHLÄGIGE VERÖFFENTLICHUNGEN[9]

Art*	Kennzeichnung der Veröffentlichung[11], soweit erforderlich unter Angabe der maßgeblichen Teile[12]	Betr. Anspruch Nr.[13]
A	Patents Abstracts of Japan, Band 5, Nr. 160 (C-75)(832), 15. Oktober 1981, & JP, A, 5692221 (ZERIA SHINYAKU KOGYO K.K.) 25. Juli 1981 --	
A	WO, A, 80/02365 (RASOR ASS. INC.) 13. November 1980 --	
A	EP, A, 0224934 (FEINSTEIN) 10. Juni 1987 --	
A	DE, A, 3637926 (SCHERING AG) 26. November 1987	

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* Besondere Kategorien von angegebenen Veröffentlichungen[10]:

"A" Veröffentlichung, die den allgemeinen Stand der Technik definiert, aber nicht als besonders bedeutsam anzusehen ist

"E" älteres Dokument, das jedoch erst am oder nach dem internationalen Anmeldedatum veröffentlicht worden ist

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"X" Veröffentlichung von besonderer Bedeutung; die beanspruchte Erfindung kann nicht als neu oder auf erfinderischer Tätigkeit beruhend betrachtet werden

"Y" Veröffentlichung von besonderer Bedeutung; die beanspruchte Erfindung kann nicht als auf erfinderischer Tätigkeit beruhend betrachtet werden, wenn die Veröffentlichung mit einer oder mehreren anderen Veröffentlichungen dieser Kategorie in Verbindung gebracht wird und diese Verbindung für einen Fachmann naheliegend ist

"&" Veröffentlichung, die Mitglied derselben Patentfamilie ist

IV. BESCHEINIGUNG

Datum des Abschlusses der internationalen Recherche	Absendedatum des internationalen Recherchenberichts
21. November 1989	10. 01. 90
Internationale Recherchenbehörde	**Unterschrift des bevollmächtigten Bediensteten**
Europäisches Patentamt	T.K. WILLIS

ANHANG ZUM INTERNATIONALEN RECHERCHENBERICHT ÜBER DIE INTERNATIONALE PATENTANMELDUNG NR.

DE 8900548
SA 30565

In diesem Anhang sind die Mitglieder der Patentfamilien der im obengenannten internationalen Recherchenbericht angeführten Patentdokumente angegeben.
Die Angaben über die Familienmitglieder entsprechen dem Stand der Datei des Europäischen Patentamts am 19/12/89
Diese Angaben dienen nur zur Unterrichtung und erfolgen ohne Gewähr.

Im Recherchenbericht angeführtes Patentdokument	Datum der Veröffentlichung	Mitglied(er) der Patentfamilie		Datum der Veröffentlichung
WO-A- 8002365	13-11-80	US-A-	4276885	07-07-81
		AU-A-	6053580	20-11-80
		CA-A-	1171952	31-07-84
		EP-A-	0028253	13-05-81
EP-A- 0224934	10-06-87	US-A-	4718433	12-01-88
		AU-B-	575735	04-08-88
		AU-A-	6609786	11-06-87
		JP-A-	62181033	08-08-87
		US-A-	4774958	04-10-88
DE-A- 3637926	26-11-87	WO-A-	8803388	19-05-88
		EP-A-	0273140	06-07-88
		EP-A-	0296189	28-12-88

EPO FORM P0473

Für nähere Einzelheiten zu diesem Anhang : siehe Amtsblatt des Europäischen Patentamts, Nr.12/82